

In the Claims

Claim 25 (currently amended) A method of operating absorbance-based chemical sensors to achieve calibration-free measurements, the method comprising the steps of:

- a) establishing wavelength accuracy to within about 2-3 nanometers;
- b) eliminating stray light at all wavelengths to about less than 0.1% incident light;
- c) preparing an analyte-selective reagent at a concentration;
- d) equilibrating the analyte-selective reagent to an analyte;
- e) taking an intensity reading of the equilibrated analyte-selective reagent and analyte at a first wavelength ( $I_{\lambda 1}$ ) with a reagent-based optical chemical sensor, wherein the sensor has been modified to allow the renewal of an analyte-selective reagent, wherein the first wavelength corresponds to an un-reacted form of the analyte-selective reagent, and taking an intensity reading of the equilibrated analyte-selective reagent and analyte at a second wavelength ( $I_{\lambda 2}$ ), wherein the second wavelength corresponds to a reacted form of the analyte-selective reagent;
- f) replacing the equilibrated analyte-selective reagent and analyte with a spectrophotometric blank solution;
- g) taking an intensity reading of the blank solution at the first wavelength ( $I_{\lambda 10}$ ), and taking an intensity reading of the blank solution at the second wavelength ( $I_{\lambda 20}$ );
- h) calculating an absorbance ratio using the equation  $A_R = A_{\lambda 1} / A_{\lambda 2}$ , where  $A_R$  is the absorbance ratio,  $A_{\lambda 1}$  is absorbance at the first wavelength and  $A_{\lambda 2}$  is absorbance at the second wavelength and, wherein  $A_{\lambda 1}$  and  $A_{\lambda 2}$  are determined by

$$A = -\log (I_x / I_{\lambda 10})$$

$$A_x = \log I_x$$

$$I_{\lambda 10}; \text{ and}$$

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- i) calculating the sensor response with the molar absorptivities ( $\epsilon$ ) of the reacted (a) form of the analyte-selective reagent and the un-reacted form (b) of the analyte-selective reagent using the equation

$$R = -\log \left( \frac{A_R - \epsilon_{\lambda 1a} / \epsilon_{\lambda 2a}}{\epsilon_{\lambda 1b} / \epsilon_{\lambda 2a} - A_R \epsilon_{\lambda 2b} / \epsilon_{\lambda 2a}} \right) = +pK_a - pH$$

~~$$R = -\log \left( \frac{A_R - \epsilon_{\lambda 1a} / \epsilon_{\lambda 2a}}{\epsilon_{\lambda 1b} / \epsilon_{\lambda 2a} - A_R \epsilon_{\lambda 2b} / \epsilon_{\lambda 2a}} \right) = +pK_a - pH$$~~

wherein when the analyte-selective reagent is prepared accurately and reproducibly at the concentration sensor readings between sensors are calibration-free.

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